

BEEF CATTLE MUSCULARITY CANDIDATE GENES

IRIDA NOVIANTI^{1,2,3}, WAYNE.S. PITCHFORD^{2,3} AND
CYNTHIA.D.K. BOTTEMA^{2,3}

¹Brawijaya University, Malang, East Java, Indonesia

²Cooperative Research Centre for Beef Genetic Technologies

³School of Animal and Veterinary Sciences, The University of Adelaide,
Roseworthy SA 5371 Australia

SUMMARY

Muscularity is a potential indicator for the selection of more productive cattle. Mapping quantitative trait loci (QTL) for traits related to muscularity is useful to identify the genomic regions where the genes affecting muscularity reside. QTL analysis from a Limousin-Jersey double backcross herd was conducted using QTL Express software with cohort and breed as the fixed effects. Nine QTL suggested to have an association with muscularity were identified on cattle chromosomes BTA 1, 2, 3, 4, 5, 8, 12, 14 and 17. The myostatin gene is located at the centromeric end of chromosome 2 and not surprisingly, the Limousin *myostatin* F94L variant accounted for the QTL on BTA2. However, when the *myostatin* F94L genotype was included as an additional fixed effect, the QTL on BTA17 was also no longer significant. This result suggests that there may be gene(s) that have epistatic effects with *myostatin* located on cattle chromosome 17. Based on the position of the QTL in base pairs, all the genes that reside in the region were determined using the Ensembl data base (www.ensembl.org). There were two potential candidate genes residing within these QTL regions were selected. They were *Smad nuclear interacting protein 1* (*SNIP1*) and similar to *follicle-stimulating-like 5* (*FSTL5*). (*JIIPB 2010 Vol 20 No 1: 1-10*)

INTRODUCTION

Muscularity can be defined as “the thickness of muscle relative to the dimensions of the skeleton” (Boer *et al.*, 1974). To select for muscularity, estimated breeding values for loin eye muscle area (EMA) adjusted to a 300kg carcass can be used (Graser *et al.* 2005). Eye muscle area is also used in calculating retail beef yield (RBY). EMA and RBY are relatively highly heritable (Koots *et al.* 1994) and estimated breeding values for these traits can be applied in selection programs to breed cattle for specific market requirements. However, many

carcass traits that are commonly selected, such as hot standard carcass weight, are not sufficient to describe the ability of the animals to produce meat since these traits are also affected by the overall size of the animal. Therefore, other muscularity traits, such as meat percentage and meat to bone ratio, would be better descriptors. Unfortunately, such traits are not usually recorded and only a limited number of quantitative trait loci (QTL) for these muscularity traits have been mapped. Finding QTL is necessary in order to identify the regions of the

genome that may contain genes affecting the traits of interest.

One gene known to have a significant role in muscle development is *myostatin*. McPherron *et al.* (1997) determined the biological function of *myostatin* by knocking out the gene in mice and demonstrating that the mutant mice were larger than the wild type mice as a result of increased muscle mass. The results proved that *myostatin* has an important role in skeletal muscle development by inhibiting muscle overgrowth.

Studies have also reported that there are many other proteins involved in the *myostatin* regulation pathway of muscle development (McPherron *et al.*, 1997; Hill *et al.*, 2002; Hill *et al.*, 2003; Lee 2004; Dominique and Gerard 2006). Therefore, there are likely to be other genes that interact with *myostatin*. The objectives of this project were to identify QTL for muscularity and related carcass traits and identified candidate genes for beef cattle muscularity based on the QTL results.

MATERIALS AND METHODS

Materials. Genotype and phenotype data from the JS Davies cattle gene mapping project were used for this study. Two breeds of cattle were used for this project, Limousin and Jersey. The two breeds (Jersey and Limousin) were chosen in the project to maximise the trait variation in the progeny from their crosses. Limousin is a beef breed of a moderate to large frame, while Jersey is a small frame dairy breed. Limousin cattle have the F94L *myostatin* genotype which affects retail beef yield (Sellick *et al.*, 2007).

The first phase on this study was conducted in 1993 by mating 280

purebred Jersey and Limousin cows to produce the first cross progeny, namely Limousin x Jersey F₁, which were born in 1994 and 1995. In the second phase, three Limousin x Jersey F₁ sires were mated to the pure Jersey and Limousin dams in Australia and New Zealand (NZ) to produce double backcross animals, called Limousin cross progeny and Jersey cross progeny herein (Sellick *et al.*, 2007). There were 161 Limousin cross progeny and 205 Jersey cross progeny born in Australia.

The phenotypic traits that were used for this study were hot standard carcass weight (HSCW), meat weight, meat percentage bone weight, bone percentage and meat to bone ratio. All traits except HSCW were estimated using regression equations from previous bone-out trials based on HSCW, fat depth, loin eye muscle area, the weight of 2-3 cuts and 2-3 bones with the protocol differing slightly for each cohort as described in Esmailizadeh *et al.* (2008). This study used the genotype data from 150 microsatellite markers in the 3 F₁ sires and their progeny.

Mapping QTL. QTL Express software (<http://qtl.cap.ed.ac.uk/>) was used to map the QTL by regression analysis of phenotypes (HSCW, meat weight, meat percentage, meat to bone ratio) and genotypes obtained from all the backcross progeny. The software is suitable for half-sib outbred populations and F₂ populations (both inbred and outbred crosses) (Seaton *et al.* 2002). A multiple marker approach for interval mapping in the half sib families was used as described by Knott *et al.* (1996) and completed at 1 cM intervals along the chromosome. Based on Knott *et al.* (1996), three steps were applied. Firstly,

informative marker alleles from the sires (361, 368 and 398) were identified to determine which allele the progeny inherited (there were 366 progeny in total) so that the sire gametes for the markers could be re-formed. On average, the sires were informative for 189 loci (Esmailizadeh, 2006). Secondly, probabilities of the individual progeny inheriting either allele 1 or 2 from the sires were calculated. Then, these probabilities were combined and provided coefficients on which the phenotypic data can be regressed. Cohort (six levels), breed (Limousin cross and Jersey cross), with and without *myostatin* F94L genotype were included as fixed effects and were nested within the sire. Three covariates were used: HSCW as a covariate for meat weight, bone weight as a covariate for meat weight and bone percentage as a covariate for meat percentage. Significant QTL were defined by selecting the QTL maxima with F-values greater than 4 as the threshold for the 3 sires families (Lander and Kruglyak, 1995). F-values greater than 4 represent $P < 0.05$ with 3 degree of freedom (for the 3 sire families).

Identification of Candidate Genes.

Using the chromosome regions that have been located using QTL Express, the positions of the markers were noted. The positions of the markers in centiMorgans (cM) were converted to base pairs (1,000,000 base pairs per centiMorgan) to identify candidate genes using the Ensembl database (www.ensembl.org). Candidate genes were chosen based on their known function or potential involvement with muscle development.

RESULTS AND DISCUSSION

QTL Mapping

QTL for all the traits (HSCW, meat weight, meat percentage and meat to bone ratio) were detected on BTA 1, 2, 3, 4, 5, 8, 12, 14 and 17 (Table 1). There were 4 QTL for hot standard carcass weight, 3 QTL for meat to bone ratio, 4 QTL for meat weight with hot standard carcass weight as a covariate, 3 QTL for meat weight with bone weight as a covariate, and 3 QTL for meat percentage with bone percentage as a covariate. Of these, 1 QTL was in common for all the traits on BTA 17. All traits except HSCW also had major QTL on BTA 2. The QTL for meat percent and meat-to-bone ratio are of particular interest as they may represent genes that specifically control muscle mass rather than just increased growth.

Table 1. Significant QTL for muscularity related carcass traits with cohort and breed as fixed effects

BTA	Traits	F-value				QTL Location (cM)			
		Nocov	Hscwcov	Bnwtcov	Bn%cov	Nocov	Hscwcov	Bnwtcov	Bn%cov
1	Hscw	4.6				87			
1	MeatWt	4.28				98			
2	MeatWt	6.08	17.27	10.96		6	6	8	
2	Meat%	20.2			17.31	6			5
2	Mttobn	9.24				8			
3	MeatWt		4.06			-	100		
3	Meat%	4.12			4.45	100			100
4	MeatWt		4.28				37		
5	Hscw	6.08				41			
5	MeatWt	4.4				32			
8	MeatWt	5.11		4.19		57		17	
12	Mttobn	4.23				31			
14	Hscw	6.74				36			
14	MeatWt	5.39				35			
17	Hscw	4.09				85			
17	MeatWt		4.87	4.84			37	82	
17	Meat%	6.07			5.11	38			38
17	Mttobn	4.42				82			

Nocov = no covariate, Hscwcov=hot standard carcass weight as covariate, Bnwt=bone weight as covariate, Bn%cov=bone percentage as covariate, Hscw=hot standard carcass weight, Meatwt=meat weight, Meat%=meat percentage, Mttobn=meat to bone ratio. Column with shade represent traits that were not analysed using specified covariate. Only significant results are noted

In order to confirm the identified QTL, a second QTL analysis was conducted which included the Limousin *myostatin* F94L genotype as a fixed effect. This QTL analysis could thus identify other chromosomal regions that might contain gene(s) that interact with *myostatin*. The QTL on BTA 1, 3, 5 and 14 were not affected by the inclusion of *myostatin* F94L genotype as a fixed effect. Since the level of significance and the location of the QTL did not change, this suggests that there are genes in these regions which control muscularity but act independently of *myostatin*. There were minor effects for the QTL on BTA 4 and 8 as the F-value slightly decreased (Table 2).

On the other hand, there were major effects of the *myostatin* genotype detected for the QTL on BTA 2 and 17. The results for BTA 2 verified that the *myostatin* F94L genotype accounted for the QTL on BTA 2. Interestingly, the meat percent QTL on BTA 17 also disappeared with the inclusion of the *myostatin* F94L genotype in the model (Figure 1). The F-values for the other QTL on BTA17 also decreased, although not as dramatically.

Since the QTL on BTA 17 represented the most of the traits of interest, a statistical analysis was conducted to confirm whether the disappearance of QTL after the inclusion of *myostatin* F94L was due to

epistatic effects or co-linearity between the *myostatin* allele and the marker alleles on BTA 17. Probabilities of 59% for the overall alleles and 99% for the sire alleles were found. This implies that the distribution was as expected.

That is, the *myostatin* allele and marker alleles were not correlated. Thus, the QTL disappearance on BTA 17 is more likely to be a consequence of an epistatic effect with *myostatin*.

Table 2. Changes in the QTL level of significance with *myostatin* F94L genotype fitted as a fixed effect with cohort and breed

BTA	Traits	F-value			
		Nocov	Hscwcov	Bnwtcov	Bn%cov
2	MeatWt	3.07	2.31	2.39	
2	Meat%	2.72			2.44
2	Mttobn	1.44			
4	MeatWt		3.49		
8	MeatWt	3.66		3.92	
17	Hscw	3.66			
17	MeatWt		2.05	3.92	
17	Meat%	2.92			2.65
17	Mttobn	3.63			

Nocov = no covariate, *Hscwcov*=hot standard carcass weight as covariate, *Bnwt*=bone weight as covariate, *Bn%cov*=bone percentage as covariate, *Hscw*=hot standard carcass weight, *Meatwt*=meat weight, *Meat%*=meat percentage, *Mttobn*=meat to bone ratio. Column with shade represent traits that were not analysed using specified covariate

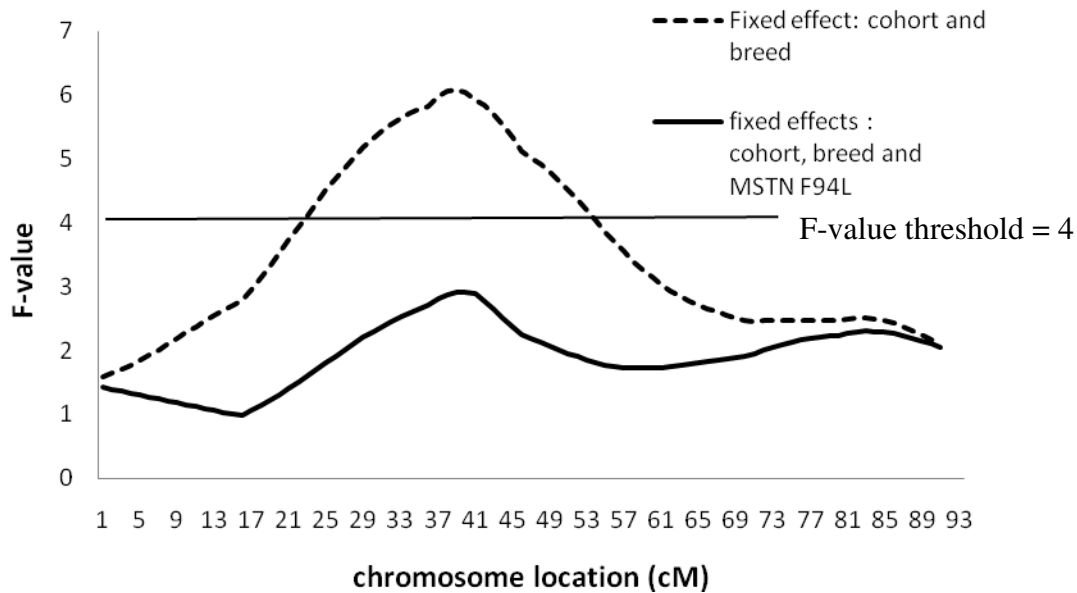


Figure 1. QTL for meat percentage on BTA 17, with and without the inclusion of MSTN F94L genotype

Candidate Genes Identification

QTL for muscularity related carcass traits were identified on BTA 2, 3, 4, 5, 8, 9, 11, 14, 17 and 18. However, the QTL found on BTA 2, 3 and 17 represented the most traits of interest. The QTL identified on BTA 2 were associated with *myostatin*, a gene known to have a major role on muscle development.

For BTA 17, the QTL for muscularity related carcass traits, such as meat weight (with HSCW as covariate) and meat percentage, also no

longer significant with the inclusion of the *myostatin* F94L genotype in the model. This indicates that there may be genes on BTA 17 that are acting epistatically with *myostatin*.

QTL on BTA 3 were significant for meat weight (with hot carcass weight as covariate) and meat percentage with or without the *myostatin* F94L genotype fitted as a fixed effect (Figure 2 and Figure 3). This result indicates that gene(s) affecting muscularity might be located on this chromosome but are unlikely to interact with *myostatin*.

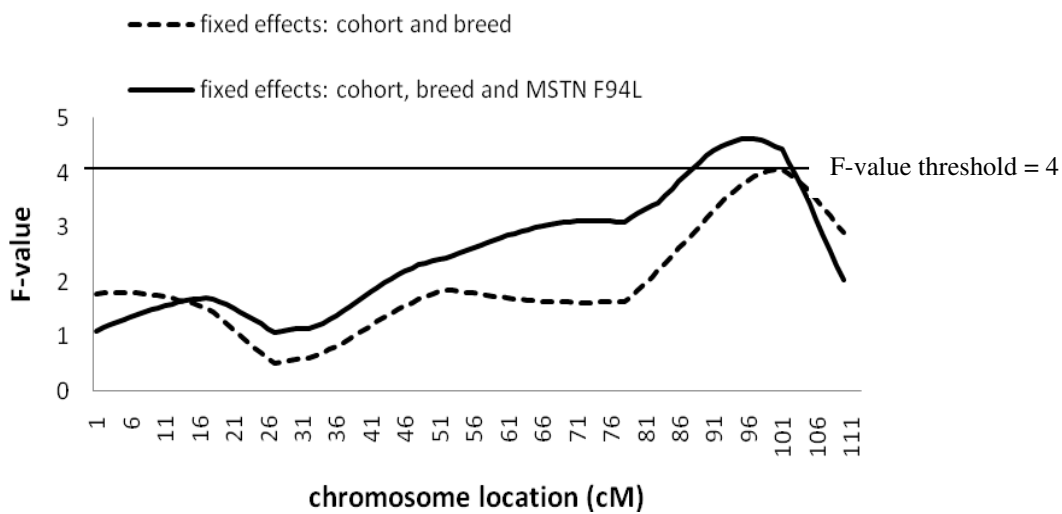


Figure 2. QTL for meat weight (with HSCW as covariate) on BTA 3, with and without the inclusion of MSTN F94L genotype

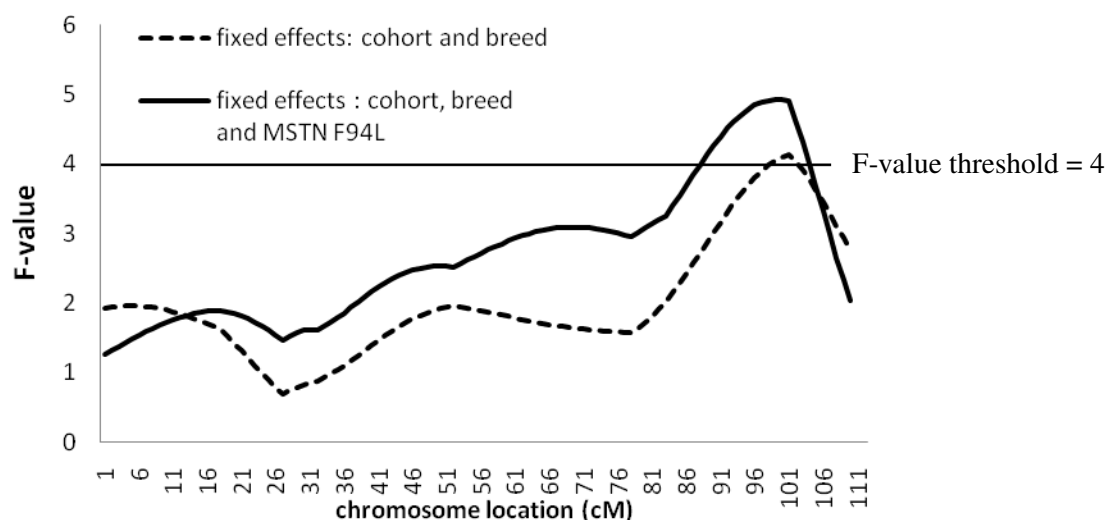


Figure 3. QTL for meat percentage on BTA 3, with and without the inclusion of MSTN F94L genotype

The relative positions (in cM) of the QTL on these two chromosomes (BTA 3 and 17) were located using microsatellite markers (Table 3). The identified markers were utilised to convert the QTL relative position from centiMorgans (cM) to base pairs (bp) using the bovine human comparative map database

(<http://www.animalgenome.org/cattle/maps/RHMap3/>). That is, the markers were used to locate the equivalent position of these QTL in the human genome sequence in addition to their position in the bovine genome sequence.

Table 3. Relative position and markers for identified QTL on BTA 3 and 17

BTA	Traits	Relative position (cM)	Markers
3	MeatWt with HSCW as covariate	100	BMS896 – BMC4214
3	Meat%	100	BMS896 – BMC4214
3	Meat% with bone% as covariate	100	BMS896 – BMC4214
17	HSCW	85	BL50 – BM1862
17	MeatWt with HSCW as covariate	37	BM941 – OARFCB48
17	MeatWt with boneWt as covariate	82	BL50 – BM1862
17	Meat%	38	BM941 – OARFCB48
17	Meat% with bone% as covariate	38	BM941 – OARFCB48
17	Mttobn	82	BL50 – BM1862
17	Silverside weight	40	BM941 – OARFCB48

MeatWt= meat weight; HSCW= hot standard carcass weight; BoneWt= bone weight, Mttobn= meat-to-bone ratio; EMA= eye muscle area

Based on the position of the QTL in base pairs, all the genes that reside in the region were determined using the Ensembl data base (www.ensembl.org). There were two potential candidate genes residing within these QTL regions were selected. They were *Smad nuclear interacting protein 1* (*SNIP1*)

and similar to *folliculin-like 5* (*FSTL5*) (Table 4). These were selected based on the function of these genes in muscle development and their association with *myostatin*. The *folliculin-like 5* gene sequence was compared to the human sequence to confirm it was homologous.

Table 4. Candidate genes list

Genes	BTA	Position (bp)
<i>Smad nuclear interacting protein (SNIP1)</i>	3	115,537,805-115,552,855
<i>Follistatin-like 5 (FSTL5)</i>	17	33,809,229-34,543,747

SNIP1 (located on chromosome 3) was selected because it is also involved in TGF- β pathway. *SNIP1* has been demonstrated to control the TGF- β signalling pathway by its interaction with the Smad proteins (Kim *et al.* 2000). Smad proteins have an important role in facilitating the signal transduction of the TGF- β family members from membrane to nucleus and in regulating the consequent changes in gene expression (Schmierer and Hill 2007).

A gene similar to *FSTL5* was found on chromosome 17 using the bovine genome sequence database. Studies have not reported the function of *FSTL5*. However, two follistatin family members, *FSTL3* (follistatin like 3/follistatin related gene) and *FST* (follistatin) are acknowledged for their contribution in the myostatin pathway (Dominique and Gérard 2006). They inhibit myostatin from binding to its receptor. Follistatin is also known to have role on muscle growth (Amthor *et al.* 1996; Amthor *et al.* 2002). Deficiency of follistatin in mice can cause muscle decrease (Matzuk *et al.*

1995). Presumably, these effects of follistatin are through its role of inhibiting myostatin. Therefore, it is suggested that *FSTL5* may have similar effect on skeletal muscle.

CONCLUSION

QTL for carcass traits related to muscularity were detected on chromosome 1, 2, 3, 4, 5, 8, 12, 14 and 17. The QTL found on BTA 2 and 17 represented the most traits of interest. The QTL on BTA 2 and 17 were no longer significant when the *myostatin* F94L genotype was included in the model. The QTL affected by the *myostatin* genotype on BTA2 were for meat weight, meat percent and meat-to-bone ratio, while the main QTL on BTA17 affected by *myostatin* was for meat percent. The results for BTA 2 verified that the *myostatin* F94L genotype accounted for the QTL on BTA 2, while the results on BTA 17 suggest that there may be gene(s) that interact or have an epistatic effect with *myostatin* for muscling on this chromosome.

Since the QTL found on BTA 3 and 17 represented the most traits of interest, two candidate genes were identified from these two chromosomes. They were *Smad nuclear interacting protein 1* (*SNIP1*) and similar to *follistatin-like 5* (*FSTL5*).

REFERENCES

- Amthor, H., B. Christ, F. Rashid-Doubell, C. F. Kemp, E. Lang and K. Patel (2002). "Follistatin regulates bone morphogenetic protein-7 (BMP-7) activity to stimulate embryonic muscle growth." Developmental Biology **243**(1): 115-127.
- Amthor, H., D. Connolly, K. Patel, B. BrandSaber, D. G. Wilkinson, J. Cooke and B. Christ (1996). "The expression and regulation of follistatin and a follistatin-like gene during avian somite compartmentalization and myogenesis." Developmental Biology **178**(2): 343-362.
- Esmailzadeh, A.K (2006) PhD Thesis. University of Adelaide.
- Esmailzadeh, A.K., Bottema, C.D.K., Sellick, G.S., Verbyla, A.P., Morris, C.A., Cullen, N.G. and Pitchford, W.S. (2008). *J. Anim. Sci.* **86**:1038
- Boer, H. D., B. L. Dumont, R. W. Pomeroy and J. H. Weniger (1974). "Manual on E.A.A.P. Reference Methods for the assessment of carcass characteristics in cattle." Livestock Production Science **1**(2): 151-164.
- Dominique, J.-E. and C. Gérard (2006). "Myostatin regulation of muscle development: Molecular basis, natural mutations, physiopathological aspects." Experimental Cell Research **312**(13): 2401-2414.
- Graser, H.U., Tier, B., Johnston, D.J. and Barwick, S.A. (2005) *Aust.J.Exp.Agric.* **45**: 913
- Hill, J. J., M. V. Davies, A. A. Pearson, J. H. Wang, R. M. Hewick, N. M. Wolfman and Y. C. Qiu (2002). "The myostatin propeptide and the follistatin-related gene are inhibitory binding proteins of myostatin in normal serum." Journal of Biological Chemistry **277**(43): 40735-40741.
- Hill, J. J., Y. C. Qiu, R. M. Hewick and N. M. Wolfman (2003). "Regulation of myostatin in vivo by growth and differentiation factor-associated serum protein-1: A novel protein with protease inhibitor and follistatin domains." Molecular Endocrinology **17**(6): 1144-1154.
- Knott, S.A., Elsen, J.M. and Haley, C.S. (1996). *Theor. Appl. Genet.* **93**:71–80.
- Koots, K. R., J. P. Gibson, C. Smith and J. W. Wilton (1994). "Analyses of published genetic parameter estimates for beef production traits. 1. Heritability." Animal Breeding Abstracts **62**(5): 309-338.
- Lander, E.S. and Kruglyak L. (1995). *Nat. Genet.* **11**: 241.
- Lee, S. J. (2004). "Regulation of muscle mass by myostatin." Annual Review of Cell and Developmental Biology **20**: 61-86.
- Matzuk, M. M., N. F. Lu, H. Vogel, K. Sellheyer, D. R. Roop and A. Bradley (1995). "MULTIPLE DEFECTS AND PERINATAL DEATH IN MICE DEFICIENT IN FOLLISTATIN." Nature **374**(6520): 360-363.
- McPherron, A. C., A. M. Lawler and S. J. Lee (1997). "Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member." Nature **387**(6628): 83-90.

- McPherron, A. C. and S. J. Lee (1997). "Double muscling in cattle due to mutations in the myostatin gene." Proceedings of the National Academy of Sciences of the United States of America **94**(23): 12457-12461.
- Schmierer, B. and C. S. Hill (2007). "TGF beta-SMAD signal transduction: molecular specificity and functional flexibility." Nature Reviews Molecular Cell Biology **8**(12): 970-982.
- Seaton G., Haley C.S, Knott S.A., Kearsley M. and Visscher P.M. (2002). *Bioinf* **18**: 339
- Sellick, G. S., W. S. Pitchford, C. A. Morris, N. G. Cullen, A. M. Crawford, H. W. Raadsma and C. D. K. Bottema (2007). "Effect of myostatin F94L on carcass yield in cattle." Animal Genetics **38**(5): 440-446.